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Göhner, Claudia; Plösch, Torsten; Faas, Marijke M

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Immune-modulatory effects of syncytiotrophoblast extracellular vesicles in pregnancy and preeclampsia

Claudia Göhner [a, b, *], Torsten Plösch [b], Marijke M. Faas [b, c]

[a] Placenta-Labor, Department of Obstetrics, Jena University Hospital, Friedrich Schiller University, Bachstraße 18, 07743 Jena, Germany
[b] Department of Obstetrics and Gynecology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713GZ Groningen, The Netherlands
[c] Immunoendocrinology, Division of Medical Biology, Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713GZ Groningen, The Netherlands

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ABSTRACT

Unique immunologic adaptations exist to successfully establish and maintain pregnancy and to avoid an immune attack against the semi allogenic fetus. These adaptations occur both locally at the maternofetal interface and in the peripheral circulation and affect the innate as well as the adaptive immune system. Pregnancy is characterized by a general inflammatory state with activation of monocytes and granulocytes, but also with suppressive lymphocytes (regulatory T cells), and skewing towards T helper 2 immunity. The pregnancy complication preeclampsia is associated with an exaggerated inflammatory state and predominance of T helper 1 and 17 immunity. The syncytiotrophoblast has been found to secrete extracellular vesicles as communication factors into the maternal circulation. Syncytiotrophoblast extracellular vesicles from normal pregnancy have been shown to interact with monocytes, granulocytes, T cells and natural killer cells and influence the function of these cells. In doing so, they may support the inflammatory state of normal pregnancy as well as the suppressive lymphocyte phenotype. During preeclampsia, syncytiotrophoblast extracellular vesicles are not only increased in numbers but also showed an altered molecular load. Based on data from *in vitro* studies, it can be suggested that syncytiotrophoblast extracellular vesicles from preeclamptic pregnancies may support the exaggerated inflammatory state during preeclampsia. In this review, we discuss the immunological functions of syncytiotrophoblast extracellular vesicles and their involvement in adapting the maternal peripheral immunological adaptations to pregnancy.

1. Introduction

Unique immunological adaptations exist to successfully establish and maintain pregnancy. These adaptations prevent an immune attack on the semi-allogenic fetus, while at the same time preserving the protection of the maternal body from external influences [1]. Therefore, local (at the maternofetal interface) and peripheral adaptations of the immune response can be found in pregnant women. Changes in the peripheral immune response are mainly characterized by activation of the innate immune system and a shift from T helper (Th) 1 and Th17 to a Th2 immune response, with increased numbers of regulatory T (Treg) cells in the adaptive immune response [2–8]. Adaptations in the peripheral immune response may be due to circulation of immune cells through the placenta and secretion of factors by the placenta into the maternal circulation, such as cytokines, placental growth factor, and soluble fms-like tyrosine kinase 1 (sFlt-1) [9,10].

Preeclampsia (PE) is a complication of the second half of pregnancy, mainly characterized by the presence of de novo hypertension and proteinuria [11]. It affects 2–8% percent of all pregnancies worldwide and accounts for the death of approximately 76,000 women and 500,000 fetuses per year [12–14]. The only known cure for PE is the termination of pregnancy which additionally increases

Abbreviations: Th, T helper cell; Treg, regulatory T cell; sFlt-1, soluble fms-like tyrosine kinase 1; STB EV, Syncytiotrophoblast extracellular vesicles; EV, extracellular vesicles; STB, syncytiotrophoblast; MV, microvesicle; NK, natural killer cell; CD, cluster of differentiation; PE, preeclampsia; NTA, nanoparticle tracking analysis; NKT, natural killer T cells; TNF-α, tumor necrosis factor α; IL, interleukin.

* Corresponding author. Placenta-Labor, Department of Obstetrics, University Hospital Jena, Bachstraße 18 07743 Jena, Germany.
E-mail address: claudia.gohner@gmail.com (C. Göhner).

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the rate of preterm birth [15,16]. PE is thought to arise from placental dysfunction [17,18], resulting in the production of pro-inflammatory factors by the diseased placenta into the maternal circulation [19–22]. Such factors may further activate the already activated innate immune response and affect the adaptive immune response during pregnancy.

One group of factors produced by the placenta in normal pregnancy and PE are syncytiotrophoblast extracellular vesicles (STB EV). Extracellular vesicles (EV) are small membrane-coated particles [23,24]. Most prokaryotic and eukaryotic cells release EV into their environment, which indicates that this evolutionary conserved process is of great importance for a cell. In the human body, diverse cells, like platelets, monocytes, endothelial cells, tumor cells but also the syncytiotrophoblast (STB), secrete EV during health and disease and EV are present in all body fluids [23,25–28]. EV are thought to have important signaling functions in many processes, for instance in immune responses. The secretion of STB EV is therefore believed to have an essential signaling role during pregnancy, for instance in the immune adaptions during pregnancy [23].

In this review, we aim to discuss the possible role of the STB EV in the immune adaptation of the peripheral immune response to pregnancy.

2. Classification and formation of extracellular vesicles

According to their size, mode of formation, function, and cellular origin, EV can be subdivided into several classes, like macrovesicles/apoptotic bodies, microvesicles (MV), exosomes, dextrasomes or prostasomes (as reviewed by Ref. [29]), but the definitions vary between researchers [30]. Most commonly, EV are subdivided into macrovesicles/apoptotic bodies, MV and exosomes [31]. Macrovesicles/apoptotic bodies are relatively large vesicles of 1–5 μm in size, which are formed by blebbing from the plasma membrane or cellular fragmentation of apoptotic cells. In the placenta, the fetal villous STB sheds macrovesicles into the maternal circulation [32]. The STB has an unique nature as being a very large multinucleated syncytium [33]. As such, the STB functions as the feto-maternal interface which regulates the exchange of nutrients and waste products between the fetal and maternal organisms. In contrast to regular macrovesicles, the placenta also sheds syncytial nuclear aggregates into the maternal circulation, which carry fetal nucleic acids [34,35]. However, placental macrovesicles including the aggregates are cleared from the maternal blood relatively fast in the maternal lungs [36]. Therefore, they will not be further object of this review. MV are 100–1000 nm in size and are formed by cells which are activated, e.g. by renewal or damage [26,37]. MV are budding directly from the apical side of the plasma membrane (Fig. 1) [26]. During this process, a translocation of phosphatidylserines from the intracellular side of the plasma membrane to the extracellular leaflet of the MV membrane may occur [26,37,38]. Phosphatidylserines are therefore often used as a specific MV marker, although MV populations have been described which do not expose phosphatidylserines on their surface (Table 1) [7].

Exosomes are small particles of 30–100 nm in size, which are formed intracellularly, inside early endosomes which are turning into intracellular multivesicular bodies [39]. The exosomes are released by fusion of the membrane of these intracellular multivesicular bodies with the plasma membrane (Fig. 1) [29,39]. Additionally, it has been described that exosome-like nanovesicles are released by budding from the cell membrane (Fig. 1) [39]. It is not clear yet, how comparable these nanoparticles are to exosomes or if they even can be differentiated from each other [29,39]. In contrast to MV, exosomes are not expected to expose phosphatidylserines on their surface. However, due to the isolation procedure and freeze-thaw-cycles, also exosome populations have been described to expose phosphatidylserines under certain circumstances [26]. Diverse molecules, such as cluster of differentiation (CD) 9, CD63, Alix, flotillin-1, and Tsg10 are enriched on exosomes and can therefore help to identify exosome-enriched fractions (Table 1) [40,41].

In general, EV are loaded with a plethora of molecules, such as proteins, (mi)RNA or DNA, which are a reflection of the current state of their originating cell as well as their communication mission [31,39]. This enables them to influence specific target cells, such as immune cells, like T cells, natural killer (NK) cells, B cells, monocytes/macrophages or dendritic cells [42,43]. Different EV subtypes from the same source may share a substantial part of their molecular cargo, however part of their molecular load is specific to the EV subset reflecting their mode of formation and their function [39,44,45].

3. Immune function of extracellular vesicles

The functions of EV have been investigated in depth and have been shown to be manifold (for a review see Ref. [39]). It has been suggested that cells secret EV to induce angiogenesis, cell survival, coagulation, waste management, cell communication and immune adaptation [23,29,39,46]. Since the molecular load of MV and exosomes differs [29,39,45], it is expected that MV and exosomes have individual functions, differing from each other.

Many studies have focused on the role of EV in immune responses. Not only do various immune cells produce EV which affect immune responses, also the immune response is influenced by EV from non-immune cells. Both immune cell EV and non-immune cell EV can induce immune stimulation, but also suppression, they can stimulate or inhibit inflammation, and they can affect autoimmune diseases and infections [39]. Immune stimulation by EV from immune cells can for instance be induced by EV from antigen presenting cells or B cells, which can directly stimulate T cells [47–49]. Immune stimulation does also take place by EV from endothelial cells which activate monocytes [50]. Moreover, EV from other cells may be able to induce immune activation, since it has been shown that autoantigens are present in the molecular load of thymocyte- or synovial fluid-derived MV and exosomes. Such particles could be involved in the pathogenesis of autoimmunity diseases such as rheumatic disease [51–53]. Next to immune activation, EV from immune cells can also induce immune suppression For instance, plasma exosomes, which are major histocompatibility complex II positive and CD11b positive (so most likely produced by monocytes or macrophages) are able to suppress immune responses in an antigen specific manner and dependent on the expression of Fas Ligand [54]. Moreover, neutrophilic EV impair the lipopolysaccharide-induced maturation of monocyte-derived dendritic cells and reduce their endocytic capacity finally resulting in an impaired inflammatory process due to failure during T cell activation [55]. Similarly, EV derived from non-immune cells may induce immune suppression. This is for instance well known for EV from tumor cells [56,57]. Exosomes isolated from ascites of ovarian cancer as well as from blood of head and neck cancer patients induced differentiation of highly suppressive regulatory T cells, as well as apoptosis of $CD8^+$ Jurkat cells [28,58]. This indicates that these EV suppress the anti-tumor immune response For advanced melanoma patients, it has been shown that MV isolated from plasma of these patients induce immunosuppressive myeloid cells

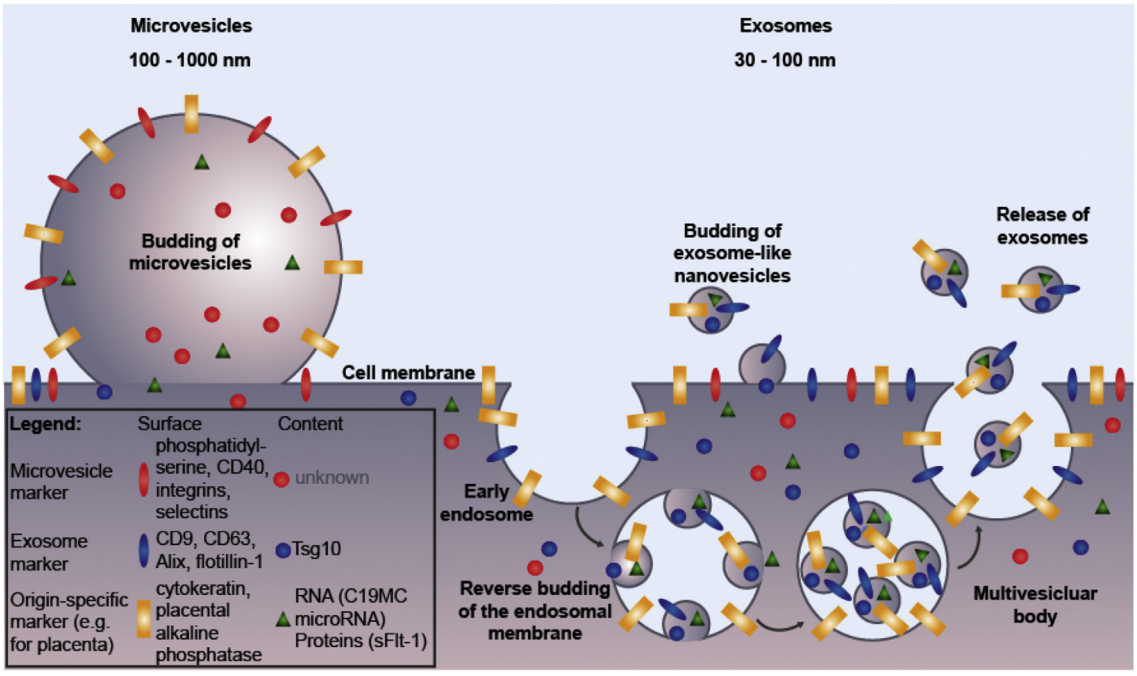

Fig. 1. Formation of microvesicles and exosomes.
Microvesicles and exosomes are formed in separate ways from their originating cell. While microvesicles bud from the plasma membrane, exosomes are built in endosomes during the formation of multivesicular bodies and released by the fusion of the membrane of the multivesicular bodies with the cell membrane. Exosome-like nanovesicles may bud directly from the plasma membrane. This different mode of formation does not only result in different vesicles sizes, with microvesicles being 100–1000 nm in size and exosomes being 30–100 nm in size. It also leads to a specific molecular load of exosomes and microvesicles while certain molecules may also be mutual. Molecules like CD9, CD63, Alix, and flotillin-1 are enriched on exosomes and can help to identify these fractions. In contrast, CD40, integrins and selectins are enriched on microvesicles and phosphatidylserines are exposed on the microvesicle surface, thus being useful to identify microvesicles. Origin-specific marker may be mutual between exosomes and microvesicles. For example, the placenta expresses cytokeratin and placental alkaline phosphatase and passes them on the STB EV. Correspondently, also the vesicle contents mirror the differences and mutuality of microvesicles and exosomes. While exosomes typically carry Trg10 in the molecular cargo, a content marker for microvesicles remains still unknown. STB microvesicles and exosomes however also feature proteins and nucleic acids of their placental origin, such as cytokeratin, placental alkaline phosphatase or the microRNA cluster C19MC.

Table 1
Overview of the types of extracellular vesicles which have been described to be relevant to pregnancy and the development of pregnancy complications.

Vesicle type	Size	Marker	Origin/biogenesis	References
Syncytial nuclear aggregates	10 to 210 μm	Condensed chromatin, cytokeratin	Clustering of nuclei which shed from the syncytiotrophoblast plasma membrane	[34,35,97,98]
Microvesicles	100 to 1000 nm	Integrins, Selectins, CD40, phosphatidylserine, placental alkaline phosphatase	Budding from the apical side of the plasma membrane, mostly from activated or damaged cells	[6,26,37,38]
Exosomes	30 to 100 nm	CD9, CD63, Alix, flotillin-1, and Tsg10, placental alkaline phosphatase	intracellularly, early endosomes turn into intracellular multivesicular bodies which fuse with the plasma membrane and release exosomes	[29,39–41]

by impairing the differentiation of monocytes into dendritic cells [59].

4. Extracellular vesicles in pregnancy and preeclampsia

In general, EV of diverse origin can be found in the circulation during pregnancy. MV have been described to originate mostly from platelets, erythrocytes, Th cells, monocytes, B cells and endothelial cells, but also STB EV were detected in the plasma of pregnant women [60,61]. Plasma concentrations of total MV were reduced at 12 weeks of gestation [60], which may reflect the disproportional increase of plasma volume compared to blood cell volume during pregnancy [62]. However, total MV concentrations recovered quickly again and subpopulations of MV secreted by monocytes, erythrocytes and STB increased [60].

Unique to pregnancy is the presence of STB EV in the circulation of pregnant women [60,63]. As indicated before, we will only focus on MV and exosomes in this review. Table 2 gives an overview of all studies which have been performed on STB EV in pregnancy and PE until now. In vitro, it has been shown that the STB releases MV and exosomes [63,64]. STB EV have been found in the circulation of pregnant women at concentrations increasing with gestational age [22]. STB MV have been found in the circulation of pregnant women from the twelfth week of gestation [22,60,63,65]. STB exosomes have been found to be increased in the maternal blood already from early pregnancy, from the sixth week of gestation, onwards [66,67].

A very comprehensive study of Tong et al. compared the molecular load of first trimester placental macrovesicles, MV and exosomes [45]. They identified over 1100 mutual proteins in all three vesicle types; however, up to 223 proteins were specific to the respective vesicle type. Most of these proteins were affiliated to physiological cellular processes, transport processes, response mechanisms or effector processes. These results indicate that although a large part of the molecular load is shared between

Table 2
Overview of the most important studies concerning syncytiotrophoblast (placental) extracellular vesicles, differences in these vesicle subtypes and their effect on normal and preeclamptic pregnancy.

Title	Main focus of the article	*In vivo*, *ex vivo*, or *in vitro*	Reference
Preparation of human placental villous surface membrane	Content on STB macrovesilces	*ex vivo*	[99]
Pre-eclampsia is associated with an increase in trophoblast glycogen content and glycogen synthase activity, similar to that found in hydatidiform moles	Content of STB microvesicles in normal and preeclamptic pregnacies	*ex vivo*	[100]
Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies	Plasma concentration of STB extracellular vesicles in normal and preeclamptic pregnacies	*ex vivo*	[68]
Trophoblast deportation in human pregnancy–its relevance for pre-eclampsia	Origin of STB macrovesicles in normal and preeclamptic pregnacies	*ex vivo*	[35]
Phagocytosis of Necrotic but Not Apoptotic Trophoblasts Induces Endothelial Cell Activation	Effects of STB macrovesicles on endothelial cells	*in vitro*	[32]
Cytoplasmic microvesicular form of Fas ligand in human early placenta: switching the tissue immune privilege hypothesis from cellular to vesicular level.	Content of STB extracellular vesicles in normal pregnancy	*ex vivo*	[101]
Syncytiotrophoblast micro-particles do not induce apoptosis in peripheral T lymphocytes but differ in their activity depending on the mode of preparation	Effects of STB extracellular vesicles on immune cells	*ex vivo*	[92]
Phosphatidylserine/phosphatidylcholine microvesicles can induce preeclampsia-like changes in pregnant mice	Effects of STB extracellular vesicles on preeclampsia	*in vivo* (mice)	[102]
The effects of apoptotic deported human placental trophoblast on macrophages: Possible consequences for pregnancy	Effects of STB macrovesciles on immune cells	*ex vivo*/ *in vitro*	[103]
Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia but not normotensive intrauterine growth restriction	Plasma concentration of STB extracellular vesicles in normal and preeclamptic pregnacies and intrauterine growth retardation	*ex vivo*	[65]
Specific isolation of placenta-derived exosomes from the circulation of pregnant women and their immunoregulatory consequences.	Effects of STB exosomes on immune cells	*ex vivo*	[104]
Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles	Effects of STB extracellular vesicles on immune cells in normal and preeclamptic pregnacies	*ex vivo*	[22]
Changes in microparticle numbers and cellular origin during pregnancy and preeclampsia	Plasma concentration of STB extracellular vesicles from divers origin in normal and preeclamptic pregnacies	*ex vivo*	[60]
T lymphocytes are targets for platelet- and trophoblast-derived microvesicles during pregnancy	Effects of extracellular vesicles from divers origin on immune cells in normal pregnacies	*ex vivo*	[90]
Phenotype and mRNA expression of syncytiotrophoblast microparticles isolated from human placenta.	Content of STB microvesicles in normal pregnancy	*ex vivo*	[105]
The effect of labour and placental separation on the shedding of syncytiotrophoblast microparticles cell-free DNA and mRNA in normal pregnancy and pre-eclampsia.	Release of STB extracellular vesicles in normal and preeclamptic pregnacies	*ex vivo*	[106]
Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function	Content of STB exosomes in normal pregnancy	*ex vivo*	[96]
Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes	Content of STB exosomes in normal pregnancy	*ex vivo*/ *in vitro*	[107]
Therapeutic effects of anticoagulant agents on preeclampsia in a murine model induced by phosphatidylserine/phosphatidylcholine microvesicles	Effects of STB extracellular vesicles on preeclampsia and potential treatment	*in vivo* (mice)	[108]
Feto-maternal interactions in pregnancies: placental microparticles activate peripheral blood monocytes.	Effects of STB microvesicles on immune cells	*ex vivo*	[88]
Morphologic and proteomic characterization of exosomes released by cultured extravillous trophoblast cells.	Content and morphology of STB exosomes in normal pregnancy	*in vitro*	[109]
Trophoblast-derived exosomes mediate monocyte recruitment and differentiation.	Effects of STB exosomes on immune cells	*in vitro*	[110]
Human trophoblast-derived exosomal fibronectin induces pro-inflammatory IL-1β production by macrophages.	Content of STB exosomes in normal pregnancy	*in vitro*	[111]
Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis.	Size characterization of STB microvesicles and exosomes in normal pregnancy	*ex vivo*	[112]
Syncytiotrophoblast microvesicles released from pre-eclampsia placentae exhibit increased tissue factor activity	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[74]
Protein composition of microparticles shed from human placenta during placental perfusion: Potential role in angiogenesis and fibrinolysis in preeclampsia.	Content of STB extracellular vesicles in normal pregnancy	*ex vivo*	[113]
The immunomodulatory role of syncytiotrophoblast microvesicles	Effects of STB microvesicles on immune cells	*ex vivo*	[46]
Trophoblast debris modulates the expression of immune proteins in macrophages: a key to maternal tolerance of the fetal allograft?	Effects of STB macrovesicles on immune cells	*ex vivo*	[114]
Syncytiotrophoblast-derived microparticle shedding in early-onset and late-onset severe pre-eclampsia	Plasma concentration of STB microvesicles in normal and preeclamptic pregnacies and their effect on endothelial cells	*ex vivo*	[69]
The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes	Content of STB exosomes in normal pregnancy	*ex vivo*	[115]
Immune cell activation by trophoblast-derived microvesicles is mediated by syncytin 1	Effects of STB microvesicles on immune cells	*ex vivo*/ *in vitro*	[116]
Heightened pro-inflammatory effect of preeclamptic placental microvesicles on peripheral blood immune cells in humans	Effects of STB microvesicles on immune cells	*ex vivo*	[72]
Immunomodulatory molecules are released from the first trimester and term placenta via exosomes	Content of STB microvesicles and exosomes in normal pregnancy	*ex vivo*	[117]
Multi-dimensional protein identification technology analysis of syncytiotrophoblast vesicles released from perfused preeclampsia placentas	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[73]
The endogenous retroviral envelope protein syncytin-1 inhibits LPS/PHA-stimulated cytokine responses in human blood and is sorted into placental exosomes	Content of STB exosomes in normal pregnancy and their effects on immune cells	*ex vivo*/ *in vitro*	[118]

Table 2 (*continued*)

Title	Main focus of the article	*In vivo*, *ex vivo*, or *in vitro*	Reference
Lipidomic analysis of human placental syncytiotrophoblast microvesicles in adverse pregnancy outcomes	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[119]
Human placental trophoblasts confer viral resistance to recipient cells	Content and anti-viral function of STB exosomes in normal pregnancy	*ex vivo*	[120]
Multicolor Flow Cytometry and Nanoparticle Tracking Analysis of Extracellular Vesicles in the Plasma of Normal Pregnant and Pre-eclamptic Women	Plasma concentration of extracellular vesicles from divers origin in normal and preeclamptic pregnacies	*ex vivo*	[70]
Hypoxia-induced changes in the bioactivity of cytotrophoblast-derived exosomes	Content and bioactivity of STB exosomes in normal and preeclamptic pregnancy	*ex vivo*	[121]
Microvesicles of women with gestational hypertension and preeclampsia affect human trophoblast fate and endothelial function	Effects of STB microvesicles on trophoblasts and endothelial cells	*ex vivo*	[122]
Exosomes secreted by human placenta carry functional Fas ligand and TRAIL molecules and convey apoptosis in activated immune cells suggesting exosome-mediated immune privilege of the fetus	Content of STB exosomes in normal pregnancy and their effect on immune cells	*ex vivo*	[94]
Characterization of syncytiotrophoblast vesicles in normal pregnancy and pre-eclampsia: expression of Flt-1 and endoglin	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[71]
Proteomic analysis of human placental syncytiotrophoblast microvesicles in preeclampsia	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[123]
Lipidomic analysis of human placental Syncytiotrophoblast microvesicles in adverse pregnancy outcomes	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[124]
Placental expression of aminopeptidase-Q (laeverin) and its role in the pathophysiology of preeclampsia	Content of STB microvesicles in normal and preeclamptic pregnancy	*ex vivo*/ *in vitro*	[125]
A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration	Content of STB exosomes in normal pregnancy and their effect on endothelial cells	*ex vivo*	[126]
Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy	Plasma concentration of STB exosomes in normal pregnacies	*ex vivo*	[66]
Trophoblast debris extruded from preeclamptic placentae activates endothelial cells: a mechanism by which the placenta communicates with the maternal endothelium	Effects of STB extracellular vesicles on endothelial cells	*ex vivo*	[127]
Plasma biomarker discovery in preeclampsia using a novel differential isolation technology for circulating extracellular vesicles	Content of extracellular vesicles from divers origin in normal pregnacies	*ex vivo*	[128]
Syncytin proteins incorporated in placenta exosomes are important for cell uptake and show variation in abundance in serum exosomes from patients with preeclampsia	Content of STB exosomes in normal and preeclamptic pregnancy	*ex vivo*/ *in vitro*	[129]
Isolation of syncytiotrophoblast microvesicles and exosomes and their characterization by multicolor flow cytometry and fluorescence Nanoparticle Tracking Analysis	Detection/characterization of STB microvesicles and exosomes in normal pregnancy	*ex vivo*	[64]
A New Enzyme-linked Sorbent Assay (ELSA) to Quantify Syncytiotrophoblast Extracellular Vesicles in Biological Fluids	Quantification of STB extracellular vesicles in liquid samples	*ex vivo*/ *in vitro*	[63]
Differential Proteomic Analysis of Syncytiotrophoblast Extracellular Vesicles from Early-Onset Severe Preeclampsia using 8-Plex iTRAQ Labeling Coupled with 2D Nano LC-MS/MS	Content of STB extracellular vesicles in normal and preeclamptic pregnancy	*ex vivo*	[130]
Antiphospholipid antibodies bind syncytiotrophoblast mitochondria and alter the proteome of extruded syncytial nuclear aggregates	Content of STB macrovesicles in normal and preeclamptic pregnancy	*ex vivo*	[131]
The Effect of Glucose on the Release and Bioactivity of Exosomes from First Trimester Trophoblast Cells	Concentration of STB exosomes and their effects on immune cells under influence of glucose	*ex vivo*	[132]
Studies of the dynamics of nuclear clustering in human syncytiotrophoblast	Release of STB macrovesicles in normal pregnacies	*ex vivo*/ *in vitro*	[98]
Isolation of human trophoblastic extracellular vesicles and characterization of their cargo and antiviral activity	Detection/characterization of STB microvesicles and exosomes in normal pregnancy	*ex vivo*	[133]
Placental exosomes and pre-eclampsia: Maternal circulating levels of normal pregnancies and early and late onset pre-eclamptic pregnancies	Plasma concentration of STB exosomes in normal and preeclamptic pregnacies	*ex vivo*	[67]
Microvesicles of pregnant women receiving low molecular weight heparin improve trophoblast function	Effects of STB microvesicles on trophoblasts and endothelial cells	*ex vivo*/ *in vitro*	[134]
Flow speed alters the apparent size and concentration of particles measured using NanoSight nanoparticle tracking analysis	Detection of STB extracellular vesicles	*ex vivo*/ *in vitro*	[135]
Proteomic characterization of macro- micro- and nano-extracellular vesicles derived from the same first trimester placenta: relevance for feto-maternal communication	Contents of STB extracellular vesicles in normal pregnancies	*ex vivo*	[45]
Trophoblast Glycoprotein (TPGB/5T4) in Human Placenta: Expression, Regulation, and Presence in Extracellular Microvesicles and Exosomes	Content of STB microvesicles and exosomes in normal pregnancy	*ex vivo*	[136]
Influence of maternal BMI on the exosomal profile during gestation and their role on maternal systemic inflammation	Plasma concentration of STB exosomes in normal and obese pregnacies and their influence on immune cells	*ex vivo*	[137]
Detection of Fetal Sex Aneuploidy and a Microdeletion from Single Placental Syncytial Nuclear Aggregates	Content of STB macrovesicles	*ex vivo*	[34]
Placental exosomes as early biomarker of preeclampsia - Potential role of exosomal microRNAs across gestation	Plasma concentration of STB exosomes in normal and preeclamptic pregnacies	*ex vivo*	[138]
Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells - Liquid biopsies for monitoring complications of pregnancy	Content of extravillous trophoblast exosomes in normal pregnacies and their effect on endothelial cells	*ex vivo*/ *in vitro*	[139]
Treating normal early gestation placentae with preeclamptic sera produces extracellular micro and nano vesicles that activate endothelial cells	Content of STB microvesicles and exosomes in normal and preeclmaptic pregnancy and their effect on endothelial cells	*ex vivo*	[140]

vesicles from the same source, respective functions are affiliated to each vesicle type based on the partially specific molecular load.

In PE, the secretion of STB EV has been shown to be significantly increased compared to normal pregnancy [65,67–69]. Other studies, however, have shown a decreased number of STB EV in preeclamptic patients [60,70]. The inconsistency between studies may be due to the methods applied. The studies that found increased numbers of STB EV in PE used an enzyme linked immunosorbent assay to measure STB EV. This method detects vesicles from all sizes. The studies that did not find increased STB EV in PE used flow cytometry. Since flow cytometry detects only larger particles, i.e. over 300 nm, this may suggest that differences in STB EV numbers between pregnant and preeclamptic women would be found in the smaller EV, i.e. below 300 nm. Indeed, a study by Tannetta et al. using nanoparticle tracking analysis (NTA), and a study by Holder et al. using electron microscopy, showed that differences in particle size between perfusates from placentae of healthy pregnant women and preeclamptic women were mainly found in the particles below 300 nm [71,72].

STB EV have not only been shown to be increased in PE as compared with normal pregnancy, but they also show a partially different molecular load than the STB EV of normal pregnancy [73]. Preeclamptic STB EV may also expose different molecules, since they expose an increased amount of tissue factor [74], and endoglin and fms-like tyrosine kinase 1 (Flt-1) [71]. This may indicate an altered functionality of STB EV during PE. However, it remains unclear how the molecular load of the vesicles is coordinated and which factors influence the differences in the molecular load of normal and preeclamptic STB EV.

5. Peripheral immune adaptions in pregnancy and preeclampsia

Pregnancy-related adaptations of the maternal immune system have been shown in both the adaptive and the innate immune response, both locally at the implantation site as well as in the peripheral circulation [75–78]. Potential local (uterine) effects of the STB EV are unknown to date. In future studies, it is worth considering whether STB EV can directly interact with uterine NK cells and monocytes/macrophages in the decidua and may be involved in uterine NK cell and macrophage function in healthy pregnancy as well as in alterations of these cell types between healthy and preeclamptic pregnancy, e.g. rather immunomodulatory/tolerance-inducing M2 macrophages in normal pregnancy vs. rather inflammatory M1 macrophages during PE [79,80]. Since the STB EV will mainly affect the peripheral immune response in pregnant women, we will discuss changes in the peripheral immune response in pregnancy and PE.

The peripheral immune response during healthy pregnancy is characterized by a generalized pro-inflammatory state [75,76,81,82]. Monocytes and granulocytes of pregnant women are not only increased in numbers but also show an activated phenotype and a different cytokine expression as compared with non-pregnant women [76,81,83]. The monocytes also show characteristics of maturation by shifting from CD16− classical monocytes to CD16+ intermediate monocytes [75,84]. Dendritic cell function seems to be suppressed to avoid an activation of the T cell response against the fetus [85]. Changes also take place in the adaptive immune response: there is an increase of Treg cells and a shift from Th1 and Th17 immunity towards type-2 immunity in T cells, NK and natural killer T (NKT) cells [2–8].

Compared to normal pregnancy, PE is associated with an exaggeration of the systemic inflammatory state characterized by endothelial and leukocyte activation [68]. It has been shown, that numbers and the activational state of monocytes and granulocytes are increased compared to normal pregnancy and that a stronger shift towards CD16+ intermediate monocytes occurs [75,76,81]. Additionally, Treg cells are reduced and Th1 and Th17 cells are predominant during PE, increasing the Th1/Th2 and Th17/Treg ratios [2,75,80,86,87]. Also, NK and NKT cells tend to produce more type-1 cytokines during PE compared to normal pregnancy [2,75,80].

6. Immune function of syncytiotrophoblast extracellular vesicles in pregnancy and preeclampsia

As indicated above, EV have many immunological functions. Indeed, evidence is growing for a role of STB EV in the immunological adaptations to pregnancy. A potential role for STB EV in the activation of the innate immune system during pregnancy has been shown by *in vitro* and *in vivo* studies indicating that monocytes can bind and internalize STB EV [22,46,88]. Due to this STB EV binding and uptake, monocytes start producing various cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL)-1-β [22,46,88]. Also, neutrophils seem to be activated by STB EV *in vitro*, since it has been shown that neutrophils incubated with STB EV produce increased levels of superoxide [89]. Our own data showed that STB EV from normal placentae not only activate monocytes (by upregulating CD11b expression) *in vitro,* but also induce maturation of monocytes by inducing a shift from classical to intermediate monocytes (unpublished).

The STB EV may also affect the adaptive immune response during pregnancy, since they have been found to bind to peripheral T and B cells and to increase signal transducer and activator of transcription 3 phosphorylation in T cells *in vitro* [46,90,91]. Furthermore, STB EV increased interferon γ production in T lymphocytes [22,92]. Additionally, STB EV are able to down-regulate proliferation of T cells induced by for instance phytohaemagglutinin [92,93]. However, the exact effect of STB EV may depend on the mode of their preparation [92]. For a better comparability of different studies with each other and to the *in vivo* situation, the preparation methods should be standardized in future studies. Additionally, the way in which STB EV interact with their target cells needs to be clarified. For example, the expression of FASL and TRAIL on STB EV may indicate that STB EV are able to induce apoptosis in T cells [94]. Furthermore, STB EV were found to have an inhibitory effect on the allogeneic reactivity of T cells in mixed lymphocyte reactions [95]. Our own results showed that STB EV isolated from perfused normal placentae may have a regulatory function: we have shown that STB EV activated Treg cells and memory T cells *in vitro* (unpublished). Although it has not been shown that STB EV can bind to NK cells, it has been shown that STB EV express ligands for the activating NK cell receptor NKG2D (MIC A/B). This is indicative for an interaction of STB EV with NK cells [96]. and indeed, STB EV can induce interferon γ production in NK cells [22], suggesting that STB EV do have an effect on NK cells. Also, different immune cell types might interact with each other and activate each other after interacting with STB EV. Future studies should thus focus on the actual interaction of STB EV with immune cells and evaluate which reactions can be attributed to direct STB EV contact and which might be a product of interaction of different immune cells.

Unfortunately, only few studies have been performed so far on the immunologic impact of STB EV from preeclamptic placentae. STB EV from explant cultures of preeclamptic placentae were able to induce increased secretion of proinflammatory cytokines such as IL1-β, IL-6, IL-17, macrophage inhibitory protein-1-α and -β, or TNF-α in peripheral blood mononuclear cells compared to STB EV from normal placenta explants [72]. Additionally, STB EV from preeclamptic placental explants increased the response of peripheral

blood mononuclear cells towards lipopolysaccharide while STB EV from normal placental explants suppressed the response of peripheral blood mononuclear cells to lipopolysaccharide [72]. This may suggest that STB EV from preeclamptic placentae may be involved in the exaggerated inflammatory response in PE. Our own study, however, showed that the effects of STB EV from preeclamptic women on monocyte activation (as measured by CD11b expression) were similar to the effects of STB EV from normal pregnant women. Our recent studies also showed that, in contrast to STB EV from normal placentae, STB EV derived from preeclamptic placentae failed to activate Treg and memory T cells (unpublished). *In vivo*, such a failure to induce a regulatory lymphocyte phenotype may enable the exaggerated inflammatory state of PE. Additional studies are needed to better align the *ex vivo/in vitro* effects seen in former studies and to compare this to the *in vivo* situation.

In conclusion, STB EV seem to represent a powerful means of communication between the placenta and the maternal body (Fig. 2). STB EV are present in the maternal circulation already from early pregnancy onwards and increase over the course of pregnancy. *In vitro* studies suggest that STB EV from healthy pregnant women can activate various inflammatory cells, such as monocytes and granulocytes, and therefore may be involved in inducing the physiologic general inflammatory state of pregnancy. Additionally, STB EV may inhibit T and NK cell functions, but induce activation of regulatory T cells and memory cells, and therefore may have a regulatory function. During PE, not only STB EV numbers are increased but also their molecular load is altered compared to normal pregnancy. Indeed, STB EV from preeclamptic pregnancies might support the exaggerated inflammatory state during PE. However, there are only a few studies addressing the immunologic function of STB EV from preeclamptic placentae. Moreover, functional studies on STB EV (both from healthy pregnancies and from preeclamptic pregnancies) have only been performed *ex vivo/*

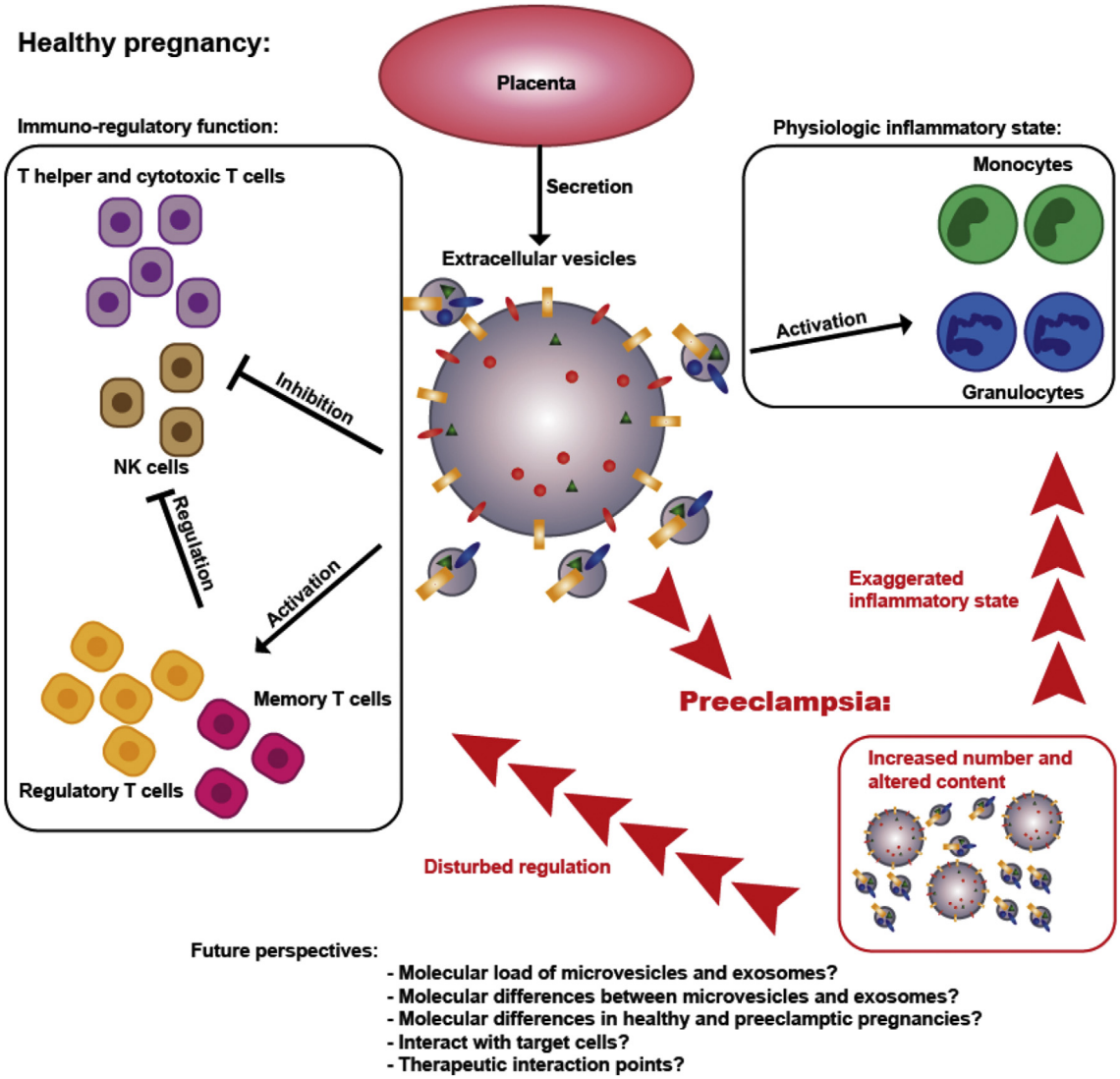

Fig. 2. The role of syncytiotrophoblast extracellular vesicles in the immunologic regulation of pregnancy.
To effectively communicate with the maternal body, the placenta secretes extracellular (STB EV) vesicles from the syncytiotrophoblast into the maternal circulation. Amongst others, the STB EV can interact with immune cells. On the one hand, they may activate monocytes and granulocytes, thus supporting the systemic inflammatory state of pregnancy. On the other hand, they may inhibit T helper and cytotoxic T cells, while promoting immuno-regulation through activation of regulatory and memory T cells. In preeclampsia, the concentration of circulating STB EV is increased and their molecular load is altered. This can support the exaggerated inflammatory state during preeclampsia and disturb immuno-regulation. However, the molecular load of STB EV in healthy and preeclamptic pregnancy, their mode of interaction with target cells and potential therapeutic interaction points remain to be established in future studies.

in vitro. Therefore, future research should focus on the *in vivo* function of the STB EV and on functional studies on STB EV, especially STB EV from preeclamptic placentae, to unravel their role in normal and preeclamptic pregnancies. This should include studies on molecular differences between STB MV and STB exosomes, but also studies into the interactions of STB EV with their target cells, e.g. by clathrin-mediated or caveolin-dependent endocytosis, micropinocytosis, phagocytosis, lipid rafts or cell surface membrane fusion. Ultimately, these studies should improve our understanding of STB EV function and open up new avenues to therapeutically interfere with STB EV signaling.

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